Aquaculture 350–353 (2012) 183–191

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Response of channel × blue hybrid catfish to chronic diurnal hypoxia

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ARTICLE INFO

Article history:
Received 15 September 2011
Received in revised form 28 March 2012
Accepted 29 March 2012
Available online 6 April 2012

Keywords:
Channel × blue hybrid catfish
Hypoxia
Dissolved oxygen
Pond production
Body compositional indices
Citrate synthase

ABSTRACT

Performance traits and metabolic responses of the channel × blue hybrid catfish (*Ictalurus punctatus* female × *I. furcatus* male) in response to chronic diurnal hypoxia were evaluated in this 197-d study. Sixteen 0.1-ha earthen ponds were stocked with 15,169 hybrid catfish/ha (47 g/fish) and managed to maintain the minimum dissolved oxygen concentration greater than 12%, 24%, 36%, or 48% of saturation. Growth and yield of channel × blue hybrid catfish were significantly related to minimum nightly dissolved oxygen concentration. The cumulative effect of nocturnal dissolved oxygen concentration and the duration of exposure to hypoxia (termed dissolved oxygen-minutes) proved to be a better independent variable for regression analysis than minimum nightly dissolved oxygen concentration. Gross and net yield and mean individual weight increased curvilinearly as dissolved oxygen-minutes increased. Chronic nightly hypoxia affected daily feed consumption and channel × blue hybrid catfish in the higher dissolved oxygen treatments grew faster because they consumed a greater percentage of their body weight at each feeding. Feed consumption increased linearly in response to dissolved oxygen-minutes during the peak production period (June–August), but curvilinearly over the entire study. Body compositional indices largely were unaffected by chronic nightly hypoxia. Lipid was the primary depot affected by hypoxia and lipid indices increased with increasing dissolved oxygen concentration and with increasing total feed fed. Citrate synthase activity was 14.6% higher in the highest compared to the lowest dissolved oxygen treatment and a strong inverse relationship between citrate synthase activity and fish body mass was observed in the highest dissolved oxygen concentration treatment. Results of this study suggest that pond dissolved oxygen concentration should be maintained at 48% saturation during the peak production period (water temperatures above 25 C) and at 36% saturation during the rest of the growing season.

1. Introduction

Farmers devote great effort to managing dissolved oxygen (DO) concentration in catfish production ponds at night during the growing season with good reason. Channel catfish (*Ictalurus punctatus*) growth and yield are affected by DO concentration and yields are higher when ponds are aerated nightly (Hollerman and Boyd, 1980; Lai-fa and Boyd, 1988). Feed intake by channel catfish reared in tank culture is reduced at DO concentrations below 70% saturation and results in reduced fish growth (Andrews et al., 1973; Buentello et al., 2000; Carlson et al., 1980). Feed consumption, growth, and yield of channel catfish grown in ponds sometimes are higher when a high (54–59% of saturation) as opposed to low DO (21–35% of saturation) concentration is maintained (Torrans, 2005, 2008). Channel catfish feed consumption and net yield decreased by 22% and 21%, respectively, when minimum pond DO concentration was maintained at 25% compared to 50% of saturation, whereas the respective decreases for the channel × blue hybrid catfish (*I. punctatus* female x *I. furcatus* male; hybrid catfish) were 10% and 10% (Green and Rawles, 2011).

Increasingly, the hybrid catfish is stocked by farmers in production ponds because the hybrid catfish is thought to perform better than purebred channel catfish in pond culture. Results of published studies are inconclusive and appear to be affected by the strain of channel catfish used as the maternal parent for the hybrid and the specific protocol of each experiment (Bosworth et al., 2004; Dunham et al., 1987, 2008; Green and Rawles, 2010; Jiang et al., 2008; Silverstein et al., 1999; Yant et al., 1976). Positive attributes reported for the hybrid catfish include faster growth (Dunham et al., 1990; Bosworth et al., 2004), greater resistance to *Edwardsiella ictaluri* infection (Wolters et al., 1996), and greater tolerance of low DO concentration (Dunham et al., 1983, 2008). Further evidence of increased tolerance of hybrid catfish to low DO concentration was presented by Green and Rawles (2011), but the optimal minimum DO concentration for pond culture was not identified. Tissue composition of hybrid catfish was not affected significantly by minimum DO concentrations of 25% or 50% of saturation (Green and Rawles, 2011), but because anaerobic protein utilization as an energy substrate increases with hypoxia in fish (Peer and Kutty, 1981) decreased tissue protein concentration may result from more severe hypoxia.

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0044-8486/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.aquaculture.2012.03.041

The objective of this study was to understand further the performance traits and metabolic responses of the channel × blue hybrid catfish in ponds where the minimum DO concentration was greater than 12%, 24%, 36%, or 48% of saturation.

2. Materials and methods

2.1. Experimental units and animals

Sixteen 0.1-ha earthen ponds located at the USDA Agricultural Research Service (ARS) Harry K. Dupree Stuttgart National Aquaculture Research Center (HKDSNARC), Stuttgart, Arkansas USA, were used for this completely randomized dose–response study. Treatments tested were minimum daily dissolved oxygen concentrations (% saturation) of 12% (DO12), 24% (DO24), 36% (DO36) or 48% (DO48). Animal care and experimental protocols were approved by the HKDSNARC Institutional Animal Care and Use Committee and conformed to ARS Policies and Procedures 130.4 and 635.1. Ponds were filled with well water in early March 2010. Salt (2241 kg/ha) was added to each pond to ensure chloride concentration exceeded 100 mg/L. Liquid fertilizer (11-37-0; average dose = 12.6 kg/ha) was added to ponds to stimulate algal production on six occasions between 5 March and 15 April. Well water was added to ponds as needed to replace losses to evaporation and seepage.

Ponds were stocked with 2009 year class fingerling channel (female) × blue (male) hybrid catfish obtained from Jubilee Farms, Indianola, Mississippi. The Jubilee strain of channel catfish was used as the maternal parent and the D&B strain of blue catfish was used as the paternal parent for hybrid fingerlings. Ponds were stocked with 15,169 ± 23 fish/ha (mean ± SD) on 30–31 March. Mean individual weight at stocking was 47 ± 4 g/fish. Ten fingerlings were selected at random, euthanized, and frozen (−80 C) until analyzed for whole body composition.

2.2. Dissolved oxygen

Dissolved oxygen (DO) concentration and water temperature in each pond were monitored continuously by a galvanic oxygen sensor (Type III, Oxyguard, Birkerød, Denmark) and thermister (Model 109, Campbell Scientific, Logan, Utah USA) connected to a datalogger (Model CR206, Campbell Scientific, Logan, Utah USA). Sensors were sampled at 10-sec intervals and mean values were outputted at 10-min intervals. We derived empirically a new variable, DO-minutes (mg min/L), from the extensive data set for each pond that was used as the independent variable in regression analysis of fish production variables:

$$\text{DO}-\text{minutes} = \sum_{i=1}^{t} DO_i * M_i,$$

where *DO* is the average DO (mg/L) during the output interval and *M* is the output interval (min), and the time period, *t*, each night. Time periods evaluated were 2000–0700 h and 0400–0600 h.

Each pond was equipped with an electric paddlewheel aerator (11.1 kW/ha; Big John Aerators, Quinton, Alabama USA) that was controlled by the datalogger to maintain DO concentration at the target level. A tractor power take-off (PTO) powered aerator (Black Cat, Aquacenter, Leland, Mississippi USA) was used as necessary to increase early morning pond DO and the duration of use was recorded. Treatment DO concentrations were maintained consistently from 16 June to 16 August, and averaged 16.3, 26.7, 37.9, and 46.4% of saturation in the DO12, DO24, DO36, and DO48 treatments, respectively. Minimum DO concentrations before and after this period were more variable in response to water temperature, but approximated treatment targets at mean early morning (0400–0600 h) water temperatures ≥25 °C.

2.3. Fish feed

Fish in each pond were fed by hand daily as much floating extruded feed (premium formulation, ARKAT Nutrition, Dumas, Arkansas USA; 32.1% protein, 6.1% lipid, 4,686.6 kcal/kg energy, 3.2% fiber, 91.3% dry matter) as they would consume in 20 min. Fish were fed once daily between mid-morning and mid-afternoon. The quantity of feed fed was recorded daily by pond. The daily feed rate expressed as a percentage of biomass was calculated for each pond as the daily feed ration divided by the estimated fish biomass on that day. Daily fish biomass was estimated by interpolation for each pond based upon stock out, sample, harvest, and mortality data.

2.4. Water quality analyses

Water samples were collected from ponds between 0700 and 0800 h with a 90-cm column sampler (Boyd and Tucker, 1992). Samples were collected on 23 March and 22 April, and weekly beginning in May. They were placed in a cooler on ice and transported to the laboratory where analyses began immediately. Chlorophyll *a* was extracted in 2:1 chloroform:methanol from phytoplankton filtered from samples using a 0.45-μ pore size glass fiber filter, and concentration in the extract was determined by spectroscopy (Lloyd and Tucker, 1988). Nitrite–nitrogen (NO_2–N, diazotization), nitrate–nitrogen (NO_3–N, cadmium reduction), and soluble reactive phosphorus (ascorbic acid method) were analyzed using flow injection analysis according to manufacturer's instructions (FIAlab 2500, FIAlab Instruments, Bellevue, Washington USA). Total ammonia–nitrogen (TAN) was analyzed fluorometrically using the *o*-phthaldialdehyde method in a flow injection system (Genfa and Dasgupta, 1989). Sample pH was measured electrometrically.

2.5. Fish sample and harvest, body compositional indices

Fish in each pond were sampled by seine net on five occasions during the experiment to monitor growth. Fish growth was sampled first on 10 May 2010 and then every 32 d, on average. At least 250 randomly selected fish per pond were weighed on each sample date. Groups of 25–50 fish were counted into a tared fish basket, weighed to the nearest 0.01 kg (Model MSI-6000, Measurement Systems International, Seattle, Washington USA), and returned to the pond. Fish were not fed the day prior to and the day of sampling.

Ponds were harvested from 12 to 15 October 2010; one replicate pond per treatment was harvested each day. Each pond was seined twice, drained, and any remaining fish were collected and weighed. At least 150 fish were selected at random from each pond and weighed individually. The total weight of fish harvested in each pond was recorded. The number of fish harvested per pond was the quotient of the total fish biomass divided by the mean individual weight. Feed conversion ratio (FCR) was calculated for each pond as the total quantity of administered feed divided by the sum of the net total yield. A random sub-sample of 20 fish from the fish sampled was collected for determination of body compositional indices (10 fish) as well as muscle and whole body composition (10 fish) by selecting every 14th and 15th fish during individual weighing. Body compositional indices included the following measures:

$$\text{Hepatosomatic index (HSI)} = \text{liver mass} \times 100/\text{fish mass},$$
$$\text{Intraperitonealfat (IPF) ratio} = \text{intraperitoneal fat mass} \times 100/\text{fish mass},$$
$$\text{Muscle ratio (MR)} = \text{skin–off fillet yield with ribs} \times 100/\text{fish mass}.$$

Muscle (hand-filleted skin-off fillets with rib cage) and whole body samples from the above procedure were immediately ground and frozen for later determination of proximate composition according to standard methods (AOAC, 2005; AOCS, 2009). Briefly, muscles or whole bodies from an individual pond were sectioned and passed through an industrial meat grinder. Ground sections were pooled for each fish, thoroughly mixed and reground two additional times before storage at −20 °C. Prior to compositional analysis, approximately 25 g frozen aliquots of ground sample from each of 10 fish/pond were thawed and thoroughly mixed as a pooled sample prior to three aliquots being taken for analysis. Moisture was determined after drying in a convection oven (Isotemp 750F, Fisher Scientific, Hanover Park, Illinois USA). Protein (N × 6.25) was determined by the Dumas method using a LECO nitrogen analyzer (FP428, LECO Corporation, St. Joseph, Michigan USA). Total energy was determined by isoperibol bomb calorimetry (Parr1281, Parr Instrument Company Inc., Moline, Illinois USA). Lipid was determined by gravimetric quantification following petroleum ether extraction (AOCS, 2009; Method AM 5-04) in an ANKOM XT15 lipid extractor (ANKOM Technology, Inc., Macedon, New York USA).

2.6. Citrate synthase activity

To assay for citrate synthase activity, a marker of aerobic respiration, white muscle samples were collected from DO48 (N = 10) and DO12 (N = 10) fish (each obtained from 4 separate ponds), snap-frozen in liquid nitrogen, and finely pulverized into powder using a liquid nitrogen-chilled spring-loaded tissue pulverizer (BioSpec Products, Bartlesville, OK). The pulverized muscle was solubilized in 100 mM Tris buffer, pH 7.4, and underwent three cycles of 10-second vortexing and liquid nitrogen/waterbath freeze-thawing in order to maximize rupturing of the mitochondrial outer and inner membranes. Samples were centrifuged at 5000 RCF at 4 °C for 10 min to yield clarified tissue lysates for both citrate synthase activity and Lowry protein assays. Citrate synthase activity was assessed as described previously by Srere et al. (1963), with modifications. Briefly, the clarified tissue lysates were added to 100 mM Tris (pH 8.0 at 30 °C.) with 0.1% v/v Triton X-100, 200 μM oxaloacetate, 100 μM acetyl CoA, and 200 μM DTNB in a total volume of 0.5 mL in a 10 mm path length cuvette. All reagents were sourced from either Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO). The change in absorbance per minute was measured at 412 nm using a Beckman DU800 spectrophotometer. Citrate synthase activity in mU/mg total protein (1 U = 1 μmol substrate converted per minute) was derived by dividing the change in absorbance per minute by 13.6 (mM extinction coefficient of TNB), multiplied by the dilution factor, and then divided by protein concentration (mg/mL) obtained from the Lowry protein assay.

2.7. Statistical analyses

Fish production, tissue composition, and compositional indices data were analyzed by mixed models analysis of variance (ANOVA) or mixed models ANOVA with repeated measures (compound symmetry covariance structure) using PROC MIXED in SAS version 9.1.3 (SAS Institute, Inc., Cary, North Carolina USA). Differences among least squares means were evaluated using the DIFF option with the Tukey adjustment of *P* values in SAS. Percent data were arcsin or log transformed prior to data analysis (Sokal and Rohlf, 1995). All dose–response data were also subjected to linear (PROC REG) and nonlinear (PROC NLIN) regression (SAS v. 9.1.3) to fit the most parsimonious model (Ratkowski, 1990). All nonlinear models belonged to the asymptotic family known as the Von Bertalanffy law in fisheries (Ratkowski, 1990). Parsimony was achieved by selecting the model with the most significant p value and highest adjusted R^2, as suggested by Kvalseth (1985), that accounts for both the number of observations (n = 16 ponds) and the number of parameters estimated (m) in each model by the following relationship:

$$\text{Adjusted } R^2 = 1 - \left\{ \left[1 - R^2\right] \left[(n-1)/(n-m-1) \right] \right\}$$

where,

$R^2 = SSreg/SStot$.
SSreg = regression sum of squares
SStot = total sum of squares
m = number of parameters estimated in the linear (m = 2), quadratic (m = 3), or exponential (m = 3) model.

Differences in citrate synthase activity between groups were tested using a two-tailed t test assuming equal variance. A Spearman rank order correlation was performed to test for a relationship between citrate synthase activity and body mass.

3. Results

3.1. Fish production and growth

Hybrid catfish gross and net yield, and individual fish weight were affected significantly by minimum DO concentration (Table 1). Pond DO concentration varied diurnally in response to photosynthesis with nightly treatment minimum DO concentrations maintained by aerator; mean diurnal DO curves are shown by treatment in Fig. 1 for the peak production period (16 June–16 August). The percentage of fish ≥0.68 kg/fish, considered a minimum market size by many processing plants, averaged 83.3%, 85.3%, 70.7%, and 57.2% for the DO12, DO24, DO36, and DO48 treatments, respectively. Fish survival did not differ significantly among DO treatments and averaged 90.9%. The relationship between net fish yield and nightly DO-minutes (2000–0700 h) was nonlinear (Fig. 2). A similar relationship was observed between gross yield (y) and DO-minutes (x) ($y = 1808.9 * \text{Ln}\ (x - 1911.5)$, $R^2 = 0.994$, $P < 0.0001$), and between mean fish weight (y) and DO-minutes (x) ($y = 130.1 * \text{Ln}\ (x - 1835.9)$, $R^2 = 0.997$, $P < 0.0001$). Nonlinear relationships also were observed between these three production variables and DO-minutes from 0400 to 0600 h.

Fish in the DO48 and DO36 treatments grew significantly faster than those in the DO24 and DO12 treatments, and fish in the DO12 treatment grew significantly slower than fish in all other treatments. Fish growth in each treatment was described by a quadratic polynomial. Regression equations were $y = 0.04066 + 0.00146x + 0.00001024x^2$ ($R^2 = 0.990$, $P < 0.0001$) for DO12, $y = 0.03393 + 0.00173x + 0.00001140x^2$ ($R^2 = 0.989$, $P < 0.0001$) for DO24, $y = 0.03911 + 0.00130x + 0.00001635x^2$ ($R^2 = 0.997$, $P < 0.0001$) for DO36, and $y = 0.03390 + 0.00166x + 0.00001504x^2$ ($R^2 = 0.993$, $P < 0.0001$) for DO48, where y is the mean fish weight (kg/fish) and x

Table 1
Least squares mean and standard error (SE) for gross and net yields (kg/ha), fish weight (g/fish), survival (%), total feed (kg/ha), and feed conversion ratio (FCR) for channel × blue hybrid catfish grown from March to October in 0.1-ha ponds where the minimum DO concentration (Min DO, % saturation) was greater than 12%, 24%, 36%, or 48% of saturation.

Treatment min DO[a]	Gross yield	Net yield	Fish weight	Survival	Total feed	FCR
12	9608 c	8911 c	721.8 c	90.2	12,844 c	1.44
24	10,800 bc	10,099 bc	805.3 b	88.2	14,741 b	1.46
36	12,574 a	11,864 a	924.8 a	92.0	17,136 a	1.44
48	12,981 a	12,280 a	933.0a	93.1	18,036 a	1.47
Pooled SE	371	371	16.7	3.1	540	0.01
Pr > *F*, ANOVA	<0.0001	<0.0001	<0.0001	0.703	<0.0001	0.425

abc Means within columns followed by the same letter do not differ significantly at the *P* value given in the table.
[a] n = 4 replicates/treatment.

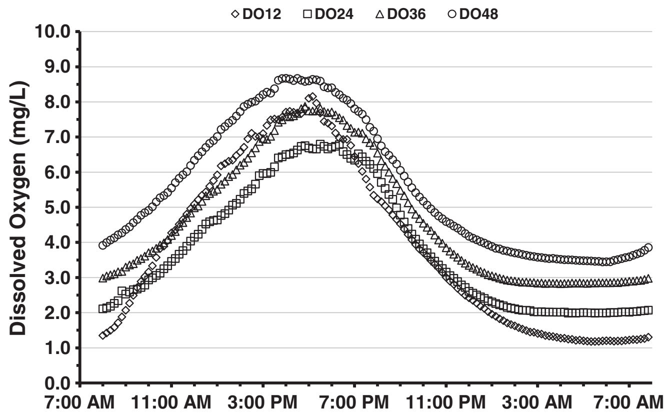

Fig. 1. Mean diurnal dissolved oxygen concentration in ponds by treatment stocked with channel× blue hybrid catfish during the peak production period (16 June–16 August). Ponds were managed to maintain minimum dissolved oxygen concentration at 12% (DO12), 24% (DO24), 36% (DO36), or 48% (DO48) of saturation.

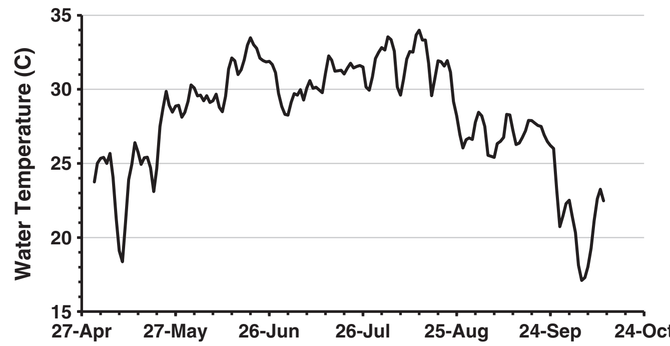

Fig. 3. Mean water temperature in ponds stocked with channel × blue hybrid catfish and managed to maintain minimum dissolved oxygen concentration at 12% (DO12), 24% (DO24), 36% (DO36), or 48% (DO48) of saturation.

is the day number. The regression equations differed significantly in the quadratic term.

3.2. Feed consumption

Fish in the two highest DO treatments consumed significantly more feed than in the two lower DO treatments (Table 1). Mean total feed consumption (y) increased nonlinearly as mean nightly DO-minutes (x) increased ($y = 2488.4 * \text{Ln}\ (x - 1930.5)$, $R^2 = 0.993$, $P < 0.0001$). Mean daily feed ration was 75, 86, 100, and 105 kg/ha for the DO12, DO24, DO36, and DO48 treatments, respectively. Fish feed consumption was high from 1 May to 25 September, declining thereafter in response to decreasing water temperatures (Fig. 3). The mean number of days mean daily feed consumption exceeded 110 kg/ha varied among DO treatments. Daily feed ration averaged 133 kg/ha for 35 d in the DO12 treatment, 141 kg/ha for 53 d in the DO24 treatment, 156 kg/d for 69 d in the DO36 treatment, and 155 kg/ha for 80 d in the DO48 treatment. Despite significant treatment differences in the amount of feed fed, no treatment differences were detected for FCR, which averaged 1.45.

Mean daily feed consumption (% biomass) by fish in each treatment during this period was inversely related to mean individual weight and there were significant differences among treatments (Fig. 4). Regression equations were: $y = 0.367 - 1.475 * \text{Ln}\ x$ ($R^2 = 0.855$, $P < 0.0001$) for DO12, $y = 0.741 - 1.386 * \text{Ln}\ x$ ($R^2 = 0.871$, $P < 0.0001$) for DO24, $y = 0.972 - 1.351 * \text{Ln}\ x$ ($R^2 = 0.856$, $P < 0.0001$) for DO36, and $y = 0.975 - 1.422 * \text{Ln}\ x$ ($R^2 = 0.880$, $P < 0.0001$) for DO48, where y is the mean daily feed consumption (% biomass/d) and x is the mean individual weight (kg/fish). The rate (slope) at which feed consumption decreased with increasing body weight did not differ among treatments. However, regression line intercepts did differ significantly among treatments. The DO12 intercept differed significantly from all other intercepts, the DO24 intercept differed from the DO48 intercept, and the DO36 intercept did not differ from the DO24 and DO48 intercepts. Thus, feed consumption by similar sized fish was higher when pond DO was maintained at higher concentrations.

The relationship between hybrid catfish daily feed consumption (% biomass) and DO-minutes from 0400 to 0600 h nightly was different at different periods during grow out. Mean daily feed consumption increased linearly as mean DO-minutes increased during the peak production (16 June–16 August) period (Fig. 5). However, over the entire experiment the relationship between mean daily feed consumption and mean DO-minutes was nonlinear (Fig. 5).

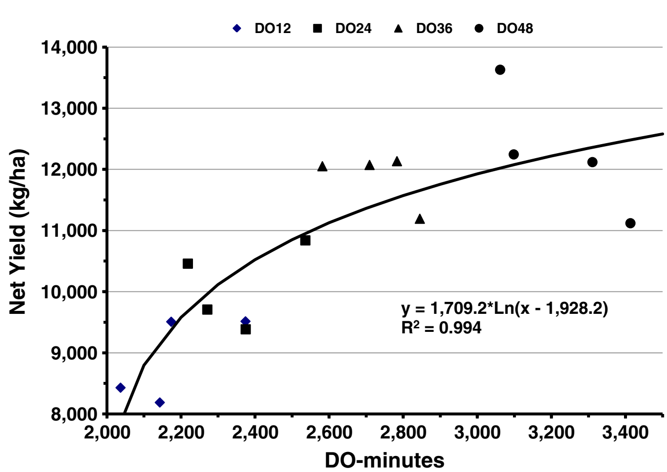

Fig. 2. Mean net yield of channel × blue hybrid catfish in relation to the mean DO-minutes from 2000 to 0700 h nightly during the period 1 May–10 October. Ponds were managed to maintain minimum dissolved oxygen concentration at 12% (DO12), 24% (DO24), 36% (DO36), or 48% (DO48) of saturation.

3.3. Aeration

Total hours of nightly aeration (y) averaged 190, 367, 678, and 1394 h (SE = 30) for the DO12, DO24, DO36, and DO48 treatments, respectively, and increased exponentially with increased minimum nightly DO concentration (x) ($y = 45.56 * \exp\ (0.8784 * x)$, $R^2 = 0.974$, $P < 0.0001$). Nightly aeration during the peak production period averaged 1.9, 3.7, 5.9, and 8.7 h/d (SE = 0.3) in the DO12, DO24, DO36, and

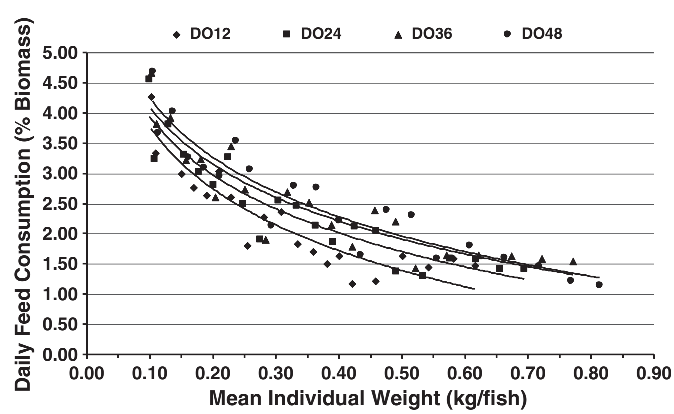

Fig. 4. Mean daily feed consumption (% biomass/d) for the period 1 May–15 September in relation to mean individual weight for channel × blue hybrid catfish grown in ponds managed to maintain minimum dissolved oxygen concentration at 12% (DO12), 24% (DO24), 36% (DO36), or 48% (DO48) of saturation. Fish were fed as much floating feed (32% protein) as they would consume in 20 min.

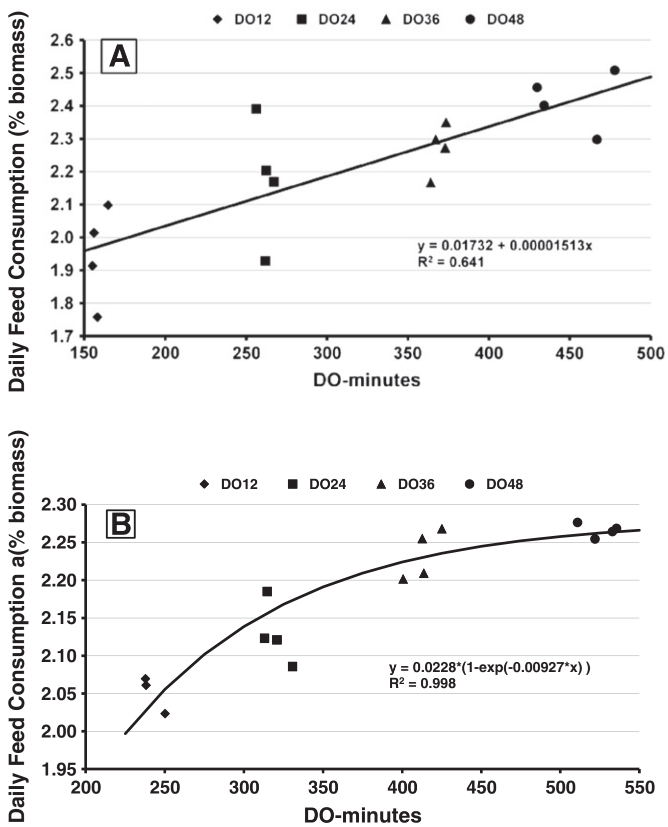

Fig. 5. Mean daily feed consumption (% biomass/d) for the period 16 June–16 August (A) and 1 May–10 October (B) in relation to the mean DO-minutes from 0400 to 0600 h nightly. Channel × blue hybrid catfish were grown in ponds managed to maintain minimum dissolved oxygen concentration at 12% (DO12), 24% (DO24), 36% (DO36), or 48% (DO48) of saturation. Fish were fed as much floating feed (32% protein) as they would consume in 20 min.

DO48 treatments, respectively, and increased linearly with increased minimum DO concentration ($R^2 = 0.945$, $P < 0.0001$). Early morning emergency PTO aeration was required on average on 20 occasions in DO48 treatment ponds; each aeration event lasted 18 min on average. Only two ponds in the DO36 treatment required emergency PTO aeration on two occasions each; mean duration of emergency aeration was 15 min/event. Mean total emergency aeration was 357 and 30 min for the DO48 and DO36 treatments, respectively. Emergency PTO aeration was unnecessary in the DO12 and DO24 treatments.

3.4. Water quality

Total ammonia–nitrogen did not differ significantly among treatments and least squares means were 0.80, 0.98, 1.10, and 1.20 mg NH_4–N/L (SE = 0.12) for the DO12, DO24, DO36, and DO48 treatments, respectively. Nitrite–nitrogen averaged 0.12, 0.24, 0.30, and 0.35 mg NO_2–N/L (SE = 0.04) in the DO12, DO24, DO36, and DO48 treatments, respectively. Only the DO12 mean was significantly less than the DO36 and DO48 means. Mean nitrate–nitrogen concentration did not differ significantly among treatments and averaged 0.16, 0.61, 0.44, and 0.89 mg NO_3–N/L (SE = 0.23) for the DO12, DO24, DO36, and DO48 treatments, respectively. Mean soluble reactive phosphorus concentration did not differ significantly among treatments; means for the DO12, DO24, DO36, and DO48 treatments were 0.03, 0.02, 0.01, and 0.03 mg PO_4–P/L (SE = 0.01), respectively. Mean chlorophyll *a* concentration differed significantly among treatments; the only significant difference was between means for the DO24 and DO48 treatments. Chlorophyll *a* concentration averaged 284.6, 226.3, 253.9, and 304.3 mg/m^3 (SE = 18.0 mg/m^3), respectively. There was a weak, positive linear relationship between mean NO_2–N and total feed fed ($R^2 = 0.477$, $P = 0.003$) and between NO_3–N and total feed fed ($R^2 = 0.317$, $P = 0.023$).

3.5. Body indices

Hybrid catfish whole body composition and retention efficiencies largely were unaffected directly by minimum DO concentration (Table 2). Whole body lipid and energy decreased linearly ($R^2 = 0.195$, $P = 0.049$) or exponentially ($R^2 = 0.256$, $P = 0.046$), respectively, with decreasing minimum DO. Positive linear relationships between whole body lipid and total feed fed ($R^2 = 0.378$, $P = 0.007$), between whole body energy and total feed fed ($R^2 = 0.287$, $P = 0.019$), and between lipid retention efficiency and total feed fed ($R^2 = 0.211$, $P = 0.042$) were an indirect effect of minimum DO concentration.

Hybrid catfish liver size (HSI; 1.17–1.25%) and muscle ratio (MR; 58.5–59.1) did not differ significantly among treatments (Table 3). Intraperitoneal fat (IPF), however, decreased linearly ($R^2 = 0.645$, $P = 0.002$) with decreasing pond DO and decreased linearly ($R^2 = 0.535$, $P = 0.0008$) with decreasing total feed fed (Fig. 6). Muscle protein content (16.1–16.8%) appeared to be unrelated to pond DO treatment (Table 3). Muscle lipid decreased linearly ($R^2 = 0.302$, $P = 0.016$) as did muscle energy ($R^2 = 0.476$, $P = 0.002$) with decreasing pond DO. Additionally, muscle lipid decreased linearly (R2 = 0.550, $P = 0.006$) and muscle energy decreased linearly (R2 = 0.676, $P < 0.0001$) with decreasing total feed fed. Muscle moisture, on the other hand, increased exponentially ($R^2 = 0.999$, $P = 0.0001$) with decreasing minimum pond DO and increased linearly ($R^2 = 0.601$, $P = 0.0003$) with decreasing total feed fed.

3.6. Citrate synthase activity

Citrate synthase activity in the white muscle of DO12 fish (112.5 mU/mg protein) was 14.6% lower in DO48 fish (131.7 mU/mg protein), however, the difference was not significant ($P = 0.385$) (Fig. 7). There was a significant negative correlation associated with citrate synthase activity and body mass in the DO48 fish (Spearman's rank correlation coefficient = −0.697; $P = 0.022$) but not in DO12 fish (Fig. 7). The regression equation was $y = 0.0008 * x^2 - 1.4341 * x + 695.92$ ($R^2 = 0.554$) (Fig. 7).

4. Discussion

In the current study, many response variables that did not differ according to analysis of variance (ANOVA) were, on the other hand, linear or nonlinear with respect to pond dissolved oxygen treatment.

Table 2
Whole body composition (fresh-weight basis) and nutrient retention in channel × blue hybrid catfish grown from March to October in 0.1-ha ponds where the minimum DO concentration (Min DO, % saturation) was greater than 12%, 24%, 36%, or 48% of saturation.

Treatment min DO[a]	Protein (%)	Lipid (%)	Energy (kcal/kg)	Moisture (%)	PRE[b]	LRE[c]	ERE[d]
12	14.8	10.4	2216	67.6	32.5	118.3	33.7
24	15.7	11.0	2333	66.0	34.4	123.6	35.0
36	15.1	11.4	2326	66.5	33.2	128.4	35.1
48	15.3	11.3	2314	66.7	33.1	124.6	34.3
Pooled SE	0.29	0.32	41.8	0.57	0.73	3.78	0.75
Pr > *F*, ANOVA	0.161	0.178	0.218	0.703	0.347	0.343	0.508

[a] n = 4 replicates/treatment.
[b] Protein retention efficiency (PRE) = protein gain * 100 / protein fed.
[c] Lipid retention efficiency (LRE) = lipid gain * 100 / lipid fed.
[d] Energy retention efficiency (ERE) = energy gain * 100 / energy fed.

Table 3
Carcass compositional indices and muscle composition (fresh-weight basis) of channel × blue hybrid catfish grown from March to October in 0.1-ha ponds where the minimum DO concentration (Min DO, % saturation) was greater than 12%, 24%, 36%, or 48% of saturation.

Treatment min DO[a]	HSI[b] (%)	IPF[c] (%)	MR[d] (%)	Protein (%)	Lipid (%)	Energy (kcal/kg)	Moisture (%)
12	1.25	5.09	58.5	16.5	6.56	1774	72.6
24	1.22	5.49	59.1	16.1	7.16	1841	72.2
36	1.17	6.00	59.1	16.6	7.28	1872	71.8
48	1.18	6.17	58.9	16.8	7.54	1913	71.1
Pooled SE	0.07	0.16	0.43	0.40	0.26	27.9	0.37
Pr>F, ANOVA	0.845	0.002	0.663	0.400	0.108	0.026	0.070

[a] n = 4 replicates/treatment.
[b] Hepatosomatic index (HSI) = liver mass × 100/fish mass; values are mean determinations on 10 fish per pond.
[c] Intraperitoneal fat (IPF) ratio = (Intraperitoneal fat mass) * 100/fish mass; values are mean determinations on 10 fish per pond.
[d] Muscle ratio (MR) = fillet mass * 100/fish mass; values are mean determinations on 10 fish per pond.

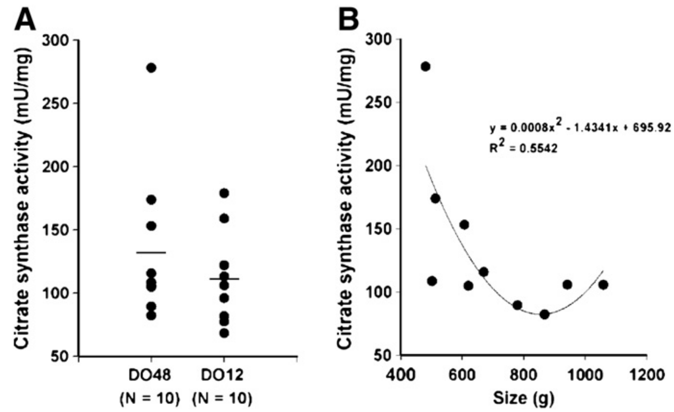

Fig. 7. A) Citrate synthase activity (mU/mg protein) in the muscle of channel × blue hybrid catfish grown in ponds managed to maintain minimum dissolved oxygen concentration at 48% (DO48) and 12% (DO12) of saturation. Solid lines indicate mean. B) Citrate synthase activity (mU/mg protein) in white muscle versus weight (grams) of DO48 fish alone.

Means separation, i.e., ANOVA, is not an optimum statistical model for dose–response data that are generated from graded levels of an independent variable. As Baker (1986) and Petersen (1977) point out, there is minimal utility in applying any kind of range or paired-comparison test to these data because the significance of differences between adjacent points is essentially meaningless, often leading to misinterpretation of results. Hence, the most relevant interpretation of results in the current study derives from the regression analyses.

Channel × blue hybrid catfish growth and yield was significantly related to minimum nightly DO concentration. However, mean nightly DO may not be the optimum independent variable to explain the production data because fish do not experience mean DO; rather, fish experience the cumulative effect of concentration and duration of exposure. Hence, we derived a new variable, DO-minutes, to describe this effect on growth, yield, and feed consumption. Thus, DO-minutes are a measure of actual cumulative exposure to nocturnal DO conditions in a pond and are analogous to the degree-days concept. Empirically derived degree-day relationships are used in aquaculture reproduction, growth, and development studies (Bry et al., 1991; Gawlicka et al., 2000; Green and Teichert-Coddington, 1993; Pawiroredjo et al., 2008; Teletchea et al., 2009).

We evaluated DO-minutes for two nocturnal time periods in the current experiment. Data points within treatment from 0400 to 0600 h were clustered more tightly than shown in Fig. 1, particularly during the peak production period (16 June–16 August), because mean pond DO, and therefore DO-minutes, varied little among ponds within treatment and was relatively constant throughout this time period. In contrast, pond DO was high at 2000 h and declined through the night until reaching the designated treatment DO concentration. Minimum nightly DO concentration generally was attained between 0300 and 0400 h. Thus, DO concentration and DO-minutes from 2000 to 0700 h varied more among ponds within treatment because of differences in pond metabolism and was reflected in the greater dispersion of data points within treatment as observed in Fig. 1. For example, the coefficient of variation in mean nightly DO concentration for the DO12, DO24, DO36, and DO48 treatments was 54.5%, 40.0%, 29.7%, and 23.3%, respectively, during the 2000–0700 h period and 2.8%, 0.5%, 0.3%, and 0.7%, respectively, during the 0400–0600 h period. Frequent DO measurements using a datalogger allowed easy calculation of DO-minutes over any time period and either of the time periods we tested yielded a strong independent variable. Computationally, calculation of DO-minutes for the 0400–0600 h time period required fewer data processing steps.

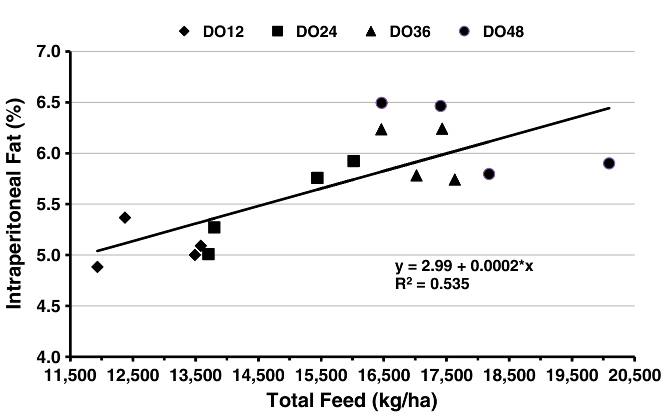

Fig. 6. Intraperitoneal fat (%) as a function of the total quantity of feed fed. Channel × blue hybrid catfish were grown in ponds managed to maintain minimum dissolved oxygen concentration at 12%, 24%, 36%, or 48% of saturation.

Chronic nightly hypoxia appeared to affect hybrid catfish growth and yield primarily by affecting daily feed consumption. Hybrid catfish in the higher DO treatments grew faster because they consumed more feed. Daily feed consumption (% biomass) by fish in the lower DO treatments began to lag compared to the higher DO treatments once treatment effects began in mid-June to be expressed consistently. The linear relationship between daily feed consumption and DO-minutes during the peak production period was further evidence of the impact of DO on feed consumption.

Only one other study reports on the relationship between feed consumption by hybrid catfish and DO concentration. In that study, Green and Rawles (2011) report that in ponds where the minimum DO concentration averaged 27.4% of saturation, hybrid catfish feed consumption and net yield each decline by 10% compared to when the minimum DO concentration averaged 49.7% of saturation. In contrast, in the current study we observed a decrease of 18% each in feed consumption and net yield when minimum DO concentration averaged 26.7% compared to 46.4% of saturation. Lower survival and a smaller initial fish size (Green and Rawles, 2011) may explain the differences between the two studies. A larger body of literature exists that addresses the effect of DO concentration on channel catfish production. Channel catfish growth is significantly lower when fish are reared under conditions of constant low compared to constant high DO concentration (% saturation) because feed intake decreases as DO percent saturation decreases and results in slower growth (Andrews et al., 1973; Buentello et al., 2000). In pond studies where DO concentration varied diurnally and minimum DO concentrations ranged from 19–37% or 54–59% of saturation, channel catfish feed consumption and net yield generally were significantly lower at the lower DO concentration (Hollerman and Boyd, 1980; Lai-fa and Boyd, 1988; Torrans, 2005, 2008). Our results for channel × blue hybrid catfish are consistent with those obtained for channel catfish.

Hybrid catfish appetite in the present study was affected by the minimum DO concentration as evidenced by feed consumption (% biomass) and the positive nonlinear relationship between total feed fed and DO-minutes. However, consumed feed was converted to biomass with equal efficiency, albeit not equal composition, in all DO treatments as indicated by the similar FCR values among treatments. Feed conversion ratio for hybrid catfish (Green and Rawles, 2011) and channel catfish (Andrews et al., 1973; Torrans, 2005, 2008) did not differ significantly between high DO (>50% saturation) and low DO (<50% saturation) rearing conditions. In ponds, catfish are exposed to diurnal fluctuations in DO concentration and generally are exposed to DO concentrations ≥ 50% saturation during daylight hours, which is when fish are fed. During the night fish can be exposed to hypoxic conditions of varying degree and duration, which was the case in the present experiment. Data available from a laboratory study on channel catfish shows that an oxygen debt accrues from anaerobic metabolism during an acute hypoxic event and is compensated for by continued hyperventilation and resultant doubling of oxygen uptake during the first hour after returning to normoxic conditions; reduced blood pH and elevated plasma lactate concentration persisted following hypoxic exposure and return to normal physiological conditions take from 2 to 6 h after return to normoxic conditions (Burggren and Cameron, 1980). This physiological response by channel catfish to hypoxia may explain the observed reduction in feed consumption following hypoxic exposure in our previous study (Green and Rawles, 2011). Hybrid catfish likely have a similar physiological response to hypoxia.

In our previous study of hybrid catfish response to low DO (Green and Rawles, 2011) we noted that no previous data existed comparing changes in whole body or tissue composition in catfish subjected to hypoxia. Moreover, prior to the current study, no data existed regarding dietary nutrient and energy retention in catfish subjected to hypoxia. As previously mentioned, minimum pond DO and feed intake are confounded in the current context and any discussion of tissue compositional changes and retention efficiencies due to hypoxia is primarily concerned with changes due to lower protein, lipid, and energy intake. Hence, similar to our previous results (Green and Rawles, 2011), the primary depot affected by restriction of feed intake as a result of hypoxia was lipid. Only body fat (IPF), and muscle and whole body lipid and energy reserves in hybrid catfish were linear or nonlinear with respect to minimum pond DO. This is not surprising given that feed consumption was linear with respect to DO from June to August, but nonlinear over the entire production season. It should be noted that, while several of the compositional responses were significantly both nonlinear and linear with respect to DO treatment, parsimony dictated that the linear relationship suffice in more cases than not. Li et al. (2005) also found linear relationships in catfish body fat, muscle fat and muscle moisture with respect to the amount fed, since the independent variable in their study, number of days fed per week, was indistinguishable from feed intake.

The range of DO treatments in our previous study in which we observed no changes in liver size (IPF), muscle ratio (MR), or protein content of whole body or fillets was 27% and 50% of saturation (Green and Rawles, 2011). Since Peer and Kutty (1981) asserted that anaerobic protein utilization as a substrate increases with hypoxia in fish, we hypothesized that both protein retention efficiency and tissue protein content would decrease with the more severe hypoxia (12% of saturation) employed in lowest treatment of the current study. Hence, we were surprised to find our current results were extremely similar to those of Green and Rawles (2011) and that there were no relationships between protein, lipid, or total energy retention and minimum pond dissolved oxygen treatment. Part of the explanation for the lack of expected differences may be due to recovery of maximum feeding and higher DO during the subsequent fall prior to harvest that may have partially ameliorated differences in retention efficiencies accrued during the depressed summer oxygen/feeding regimes.

To better understand the metabolic mechanisms underlying the response of hybrid catfish to hypoxia, we investigated the activity of citrate synthase in the white muscle of fish sampled from the highest and lowest DO treatments. The activity of citrate synthase, the enzyme catalyzing the first step of the citric acid cycle, serves as a key indicator of aerobic metabolism and has been shown to decrease in tissues of fish subjected to both acute and chronic hypoxia (Cooper et al., 2002; Zhou et al., 2000). Although the 14.6% reduction in citrate synthase activity observed in DO12 fish as compared to DO48 was not statistically significant, the reduction may have biological relevance and may represent a diminution of aerobic respiration and a metabolic shift to more anaerobic metabolism in order to meet energy requirements. Furthermore, the absolute differences in citrate synthase activity between DO12 and DO48 treatments were made smaller by the strong inverse relationship observed with body mass and citrate synthase activity in DO48 fish. This allometric scaling of citrate synthase, in addition to other metabolic enzymes, has been demonstrated in a number of fish species, but never in hybrid catfish. The depression in citrate synthase activity in larger fish in the DO48 treatment may have resulted from the increased food intake and growth observed in DO48 ponds. Increased food intake can markedly increase postprandial oxygen consumption (specific dynamic action) (Andrews et al., 1973; Buentello et al., 2000; Secor, 2009) and size has been shown to be an important determinant of metabolism as larger fish tend to rely more heavily on anaerobic pathways due to factors such as increased blood circulation time and concomitant decreased rates of oxygen delivery to respiring tissues (Cooper et al., 2002). Specific dynamic action (SDA) refers to the energy expended by an organism to consume, digest, and assimilate a meal and is affected by environmental factors (including hypoxia) and characteristics of the fish and the meal (Secor, 2009). Protein synthesis is considered to be the putative dominant process affecting SDA (Secor, 2009). Studies are ongoing in our laboratory to examine the activity and expression of other metabolic enzymes and genes that may be important in the response of hybrid catfish to chronically low DO. From these studies we hope to develop assays predictive of fish performance and the ability to cope with hypoxic stress encountered in production settings.

The fact that changes in muscle composition were generally more severe, however, than those in whole body suggests that catfish spared protein and lipid in organs at the expense of body fat and muscle lipid reserves. These results also suggest that catfish, similar to other animals, maintain muscle and organ protein at the expense of lipid during underfeeding via interorgan coordination of the glucose/fatty acid/ketone body cycle. In this scheme, mobilization of lipid reserves and subsequent fatty acid oxidation to ketone bodies is preferential to protein and concomitantly inhibits flux of glucose or amino acids through the Krebs cycle (Wu and Marliss, 1992). Hence, our observation that citrate synthase activity was depressed in the lowest DO treatment is also consistent with interorgan regulation of energy substrate utilization during hypoxia-induced underfeeding. One advantage of this metabolic scheme during hypoxia would be that acetyl CoA derived from β-oxidation of fatty acids enters the HMG-CoA cycle for the synthesis of ketone bodies that can subsequently be used for energy by several organs in place of glucose or amino acids. Moreover, though oxygen is consumed in the formation of ketone bodies, there is no production of CO_2. In addition, while carbohydrate is not a major energy substrate in fish, muscle glycogen represents a putative energy source that can be mobilized during hypoxia (Heath and Pritchard, 1965; Sanger, 1993). While muscle glycogen was not measured in the current study, the observations that muscle protein remained constant, while muscle lipid declined linearly and muscle moisture increased exponentially with decreasing DO, suggests that glycogen was the additional decreasing component of muscle composition that resulted in exponential, rather than linear, increases in moisture in fish subjected to the greatest hypoxia.

Pond water quality throughout the experiment was unremarkable and was consistent with water quality reported for other hybrid or channel catfish grow out pond trials (Green and Rawles, 2010, 2011; Li et al., 2010; Torrans, 2005, 2008). The quantity of feed fed is a main determinant of pond water quality, and in the present experiment the quantity of feed fed increased nonlinearly as minimum DO concentration increased. However, large quantities of feed were fed in all treatments, which would explain the general absence of treatment differences in water quality variables and the weak, positive linear relationship between NO_2–N and NO_3–N and total feed fed.

In summary, hybrid catfish performance was affected negatively by exposure to chronic diurnal hypoxia. In fact, the relationships observed between feed consumption and DO-minutes for the peak and entire production periods suggest a two-stage pond DO management strategy may be justified: maintain DO concentration at 48% saturation during the peak production period, which was when water temperatures exceeded 25 C, and at 36% saturation during the rest of the production period. Clearly, such a strategy must be validated technically and economically in commercial ponds before being recommended formally.

Acknowledgments

We thank Greg O'Neal and Rebecca Jacobs for their assistance throughout this study, Dr. Adam Fuller, Matt McEntire, and Bradley Farmer for their assistance with tissue sample collection, and our many co-workers at the Stuttgart National Aquaculture Research Center who assisted during harvest. We also thank the UCDC DRTC BARB Core (P60 DK079626) at the University of Alabama at Birmingham for examining citrate synthase activity. This study was funded by USDA/ARS under project number 6225-31630-006-00D. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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